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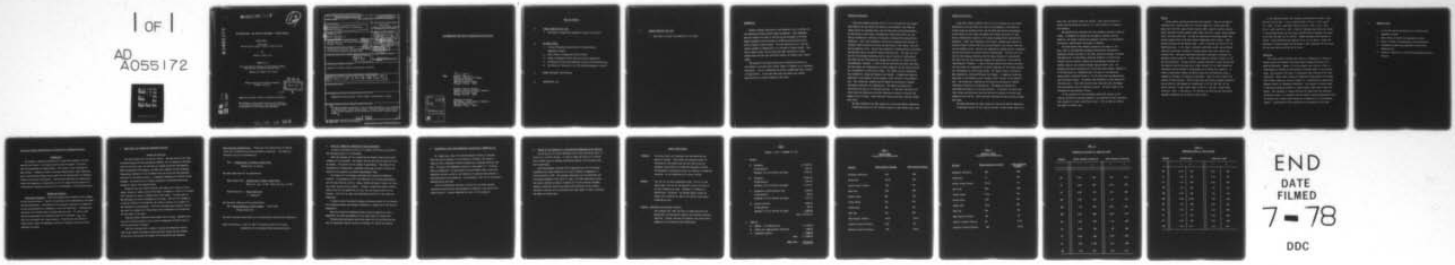

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THE MANUFACTURE AND STUDY OF HEMOGLOBIN - SALINE SOLUTION

Final Report

April 1978
(for the period July 1, 1976 to June 30, 1977)

by

Gerald S. Moss, M. D.

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

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Hektoen Institute for Medical Research
Chicago, Illinois

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Primates exchange transfused to 6 gms % native hemoglobin can be stimulated to increase their cardiac output by 60%. Primates exchange transfused to 6 gms foreign hemoglobin can be stimulated to raise their cardiac output by 40%.

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THE MANUFACTURE AND STUDY OF HEMOGLOBIN-SALINE SOLUTION

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A. STUDIES COMPLETED THIS YEAR

1. The Effect of Stroma Free Hemoglobin on the Heart

INTRODUCTION

Primates exchange transfused to zero hematocrit with a modified red cell hemolysate maintain normal oxygen consumption. Their hemoglobin mass was reduced to 6 grams %, and the P_{50} was decreased to 14 mm Hg. Oxygen consumption was maintained by increasing the extraction ratio, with no change in cardiac output. This was surprising to us since the typical response to hemodilution is an increase in cardiac output. Some form of hypoxic heart failure could have been responsible or these animals might not have had sufficient stimulus to increase their cardiac output.

The purpose of the current study was to evaluate the ability of such animals to increase their cardiac output in response to a β-adrenergic stimulation. This was accomplished by using a standardized bolus infusion of isoproterenol. It has been shown that the hypoxic and ischemic myocardium has an altered response to this drug.

METHODS AND MATERIALS

Eight adult baboons weighing from 13.5 to 21.8 kg were the test animals. Approximately ten days before the study an electromagnetic flow probe was placed around the ascending aorta, and the leads were buried subcutaneously. On the morning of each study, the baboon was tranquilized within its cage with an intramuscular injection of 0.8 mg/kg of phencyclidine hydrochloride piperazine. Under local anesthesia, four plastic catheters were inserted in bilateral femoral arteries and veins and positioned in the inferior vena cava and the abdominal aorta. One pair was connected to Statham pressure transducers and monitored aortic and central venous blood pressures. The other pair was used for the exchange transfusion and for venous infusion of isoproterenol. The leads from the flow probe were exposed and connected to a Micron RC-100 electromagnetic flowmeter. A lead II EKG was obtained with needle electrodes. The flow signal was integrated by an analog integrator and the output analyzed by a minicomputer to yield stroke volume. The signals from all transducers were recorded on a Brush multichannel oscillograph. A temperature probe was positioned in the abdominal cavity through a small incision in the abdominal wall. The trachea was intubated and the baboon was paralyzed by frequent intravenous injections of d-tubocurarine. The baboon was mechanically ventilated with room air in the prone position. In the base line period the tidal volume and respiratory rate were adjusted to produce an arterial pCO_2 between 33 and 47 mm Hg. These ventilator settings were held constant through each study.

The body temperature was kept constant by raising the ambient temperature.

A continuous monitor of CVP, arterial pressure, stroke volume, heart rate,

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The body temperature was kept constant by raising the ambient temperature.

A continuous monitor of CVP, arterial pressure, stroke volume, heart rate,

heart rate, and cardiac output was started. After several minutes of control data the animal was given an I.V. bolus infusion of 2 mcg/kg of isoproterenol.

The monitoring was continued until the variables returned to baseline values. A minimum of 45 minutes was provided for recovery from the injection. The animals received two subsequent infusions of isoproterenol. The protocol required about three hours to complete.

The eight baboons were randomly assigned to two groups of four each. Those in one group were exchange transfused with the modified hemolysate solution and those in the other group were exchange transfused with Dextran-75. In each baboon, blood was withdrawn in 50 milliliter aliquots from the arterial catheter and simultaneously replaced by a similar volume of test solution infused into the femoral vein. The exchange transfusion continued until the Dextran group reached a hematocrit of 20 (equivalent to a hemoblobin mass of 6 grams %) and hemolysate group reached a hematocrit below 2. At this point they had approxmiately equal hemoglobin masses. The animals were maintained at these hematocrits for three hours. After this period of time, they again were challenged with isoproterenol using an identical protocol. The total length of the procedure was approximately 12 hours.

At the conclusion of each experiment statistical analysis of the data was performed to determine whether or not additional animal experiments were necessary to reach a significant result. This was done to minimize the number of animals used.

RESULTS

Eleven primary and derived variables were analyzed. They are: peripheral resistance (PR), cardiac output (CO), stroke volume (SV), cardiac work (CW), peak aortic flow (PF), mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), heart rate (HR), central venous pressure (CVP), and stroke work (SW). All data was expressed as percentage change from the pre-isuprel state to the peak response immediately after isuprel. Table I contains the data for the Dextran group. Table II exhibits the results for the hemolysate group. In all cases a consistent qualitative pattern was exhibited. There were significant increases in cardiac output, heart rate, cardiac work and peak flow. There were significant decreases in peripheral resistance and diastolic arterial pressure. Stroke volume tended to increase slightly, as did CVP and stroke work. The mean arterial pressure exhibited a slight decrease and the systolic arterial pressure had a slight increase. All of these changes are consistent with the concept of isoproterenol as an almost pure β-agonist. In order to quantitavely compare the Dextran group with the hemolysate group, a sequence of analyses of variances was performed. Table III and IV contain the results of these analyses. Table IV shows that the Dextran exchange transfusion had no effect on the response to isoproterenol of SV, CO, DAP, MAP, SW, CW, PR, PF, and CVP. It had a small effect on SAP ($p = .04$) and a larger effect on HR ($p = .012$). If we used a $p < .01$ criteron, we could say that the Dextran exchange transfusion had no effect on any variable.

In the hemolysate group, the exchange transfusion had no effect on DAP, SAP, MAP, CW, and CVP. It had a moderate effect on SV ($p = .013$), and PF ($P = .044$). It had a significant effect on HR ($p = .001$), CO ($p = .005$), SW ($p = .007$), and PR ($p = .003$). Table III indicates that during the control or pre-exchange period, the two groups exhibited similar responses with report to CO, DAP, SAP, MAP, CW, PR, and CVP. Slight differences were observed in the SW. Large differences were observed in the HR, SV, and PF. Hence, the difference in pattern between the two groups is most consistent for the CO and PR, and less clear for the HR, SW, PF and SV.

Discussion

This study clearly indicates that there is a diminuation of response in cardiac output and peripheral resistance after exchange transfusion with a modified hemolysate. However, it is by no means clear that this effect is large enough, or relevant to the constancy of cardiac output in our previous study. The increase in CO, after β-stimulation, went from 62% to 44% after transfusion. Hence, after 3 house at 0 hematocrit these animals can increase their cardiac output by a factor of almost 1.5. We believe this demonstrates adequate reserve for adrenergic stimulation. The increase in cardiac output in the Dextran group was primarily a stroke volume, rather than a heart rate effect. Such increases in stroke volume are not associated with adrenergic stimulation; hence, it is possible that the animals receiving hemolysate failed to increase their cardiac output because of a depression of a non-adrenergic channel. A description of this possibility is not possible at this time.

B. ONGOING STUDIES

1. In Vivo Off-Loading Characteristics of Reconstituted Hemoglobin Solution
2. Renal Effects of Stroma Free Hemoglobin Solution
3. Effect of Stroma Free Hemoglobin on Brain Oxygenation
4. Collaborative Study Using Hemoglobin Solution with Normalized P_{50}
5. Effects of Infusion of 2,3 DPG Enriched Hemoglobin-Saline Solution

IN VIVO OFF-LOADING CHARACTERISTICS OF RECONSTITUTED HEMOGLOBIN SOLUTION

INTRODUCTION

The shipment, storage and handling of a stroma-free hemoglobin solution would be facilitated if its weight and bulk could be reduced. In the past, this was accomplished by lyophilization. The success of this technique has been limited. Although we intend to evaluate lyophilization, other techniques such a ultrafiltration might also be useful for the concentration of hemoglobin solution and require investigation. Once the best technique for concentrating stroma free hemoglobin is determined, the in vivo oxygen carrying capacity of "reconstituted" hemoglobin solution will be investigated in the following manner.

METHODS AND MATERIALS

Preliminary Procedures: Adult male baboons having bilateral groin cutdowns will be the test animals. They will be paralyzed with d-tubocurarine, ventilated and kept normothermic. The animals will be exchange transfused to a hematocrit of zero and maintained at a hematocrit of zero for three hours. Following this, the animals will receive their own washed shed red cells. At various times during the experiments the following values will be obtained: P_{50}, Po_2, Pco_2, pH, and jugular vein oxygen contents. From this data the ability of reconstituted stroma free hemoglobin solution to transport O_2 and CO_2 can be adequately evaluated.

2. RENAL EFFECTS OF STROMA-FREE HEMOGLOBIN SOLUTION

METHODS AND MATERIALS

Five adult baboons will be the test animals. One week prior to the study, siliconized heparin-filled polyethylene catheters will be surgically implanted into the left renal vein, and the free end ligated and buried subcutaneously. After convalescence from surgery, the renal vein catheter will be re-exposed. Polyethylene catheters will be introduced into the aortic arch and suprarenal vena cava via the femoral vessels. A suprapubic cystotomy will provide urinary drainage. The animals will be loosely restrained in the prone position and allowed to recover from tranquilization.

During the four hour baseline period, the animals will receive an intravenous infusion of normal saline with 44 mEq/L of $NaHCO_3$ at a rate of 10 cc/kg/hr. This infusion will insure a constant basal urine output. A primary dose of PAH (40 mg/kg) and Inulin (75 mg/kg) will be given. This will be followed by a constant infusion of 0.5% PAH and 1.0% Inulin in saline, at 6 cc/kg/hr for the remainder of the experiment. At 60 and 75 minutes after priming, arterial and renal vein samples and fifteen minute urine collections will be obtained for the course of the study.

Every 30 minutes thereafter blood samples will be taken. Beginning with the first 30 minutes period, a 50 cc bolus of hemoglobin solution (6 gms %) will be given every 15 minutes.

This will continue until a volume of stroma-free hemoglobin solution (50% of the animals calculated circulating blood volume) has been infused. At the end of this period the animals will be sacrificed and autopsied.

Renal Clearance Determinations: Plasma and urine concentrations of PAH and Inulin will be determined by spectrophotometric techniques. The glomerular filtration rate will be determined by:

$$GFR = \frac{\text{(Urinary Conc. of Inulin) x (Urine Flow)}}{\text{(Plasma Conc. of Inulin)}}$$

The renal blood flow will be determined by:

$$\text{Renal Plasma Flow} = \frac{\text{(Urinary Conc. of PAH) x (Urine Flow)}}{\text{(Renal Art. Conc. of PAH - Renal Vein Conc. of PAH)}}$$

$$\text{Renal Blood Flow} = \frac{\text{Renal Plasma Flow}}{1 - \text{Hematocrit}}$$

The Free Water Clearance will be determined by:

$$FWC = \frac{\text{(Urine Osmolarity x (Urine Volume)}}{\text{(Plasma Osmolarity)}} - \text{Urine Volume}$$

The urine and plasma osmolarities will be determined by freezing point depression.

Renal A-V Shunting: To test for renal A-V shunting, blood will be drawn anerobically for simultaneous blood gas determinations.

3. EFFECT OF STROMA-FREE HEMOGLOBIN ON BRAIN OXYGENATION

In order to evaluate the effect of a leftward shifted P_{50} on the brain, the following study will be undertaken:

Adult male baboons will be tranquilized and femoral venous and arterial cutdowns will be performed. One jugular vein and one carotid artery will also be cutdown. The animals will be allowed to equilibrate. Then they will be paralyzed with d-tubocurarine, intubated, ventilated and their arterial Po_2, and pH will be adjusted, and baseline measurements taken.

The animals will be exchange transfused with stroma-free hemoglobin solution. At decrements of 10% in the hematocrit the following measurements will be taken from the arterial, venous, carotid and jugular routes: pH, Pco_2, Po_2, serum lactates and O_2 contents. Through an indwelling catheter cerebrospinal fluid will be sampled and pH, Pco_2, Po_2 and lactate levels will be measured. EEG's will be taken each measurement period after the animal has stabilized.

A control group of Dextran-75 exchange transfused animals will be handled in an analogous manner and exchange transfused to a hematocrit of 20% (6 gms % hemoglobin).

When the stroma-free hemoglobin animals reach 0% hematocrit (6 gms % hemoglobin) the above measurements will be taken hourly for three hours.

Following the experimental period the animal will be sacrificed and the horn of hippocampus removed and sent to pathology for section and viewing.

4. COLLABORATIVE STUDY USING HEMOGLOBIN SOLUTION WITH A NORMALIZED P_{50}

Dr. Thomas Zuck, Chief of the Blood Research Division at Letterman Army Institute of Research in San Francisco, California, has agreed to supply us with a hemoglobin solution prepared by a technique different than ours. The P_{50} of this hemoglobin solution is more normal (17 - 19 mm Hg) than our preparation. We have agreed to use our baboon model to test this hemoglobin solution as soon as the logistics of supplying large amounts of this preparation can be worked out. We anticipate a start up for the project sometime during this year.

The first experiments using this solution will be simple exchange transfusions measuring the same parameters as measured in our earlier work (refer Section A - Studies Completed this Year, of this proposal).

5. EFFECTS OF THE INFUSION OF 2,3 DPG ENRICHED HEMOGLOBIN-SALINE SOLUTION

The low P_{50} of our current hemoglobin-saline casts some doubt about its utility in a clinical setting. In order to study the effect of a solution with a higher P_{50} as an exchange transfusion material, the following study will be performed.

Adult male baboons, paralyzed with d-tyborcurarine, will be exchange transfused with either Dextran-75 or a 6 gm % solution of hemoglobin in saline enriched 2,3 DPG. The exchange transfusion will be halted when the circulating hemoglobin level falls to 6 gms % in either experimental group. The cardiac response to Isuprel injection will be the principal parameter measured, along with arterial and venous blood pressures and O_2 contents. Statistical analysis will be performed after each animal in order to minimize the number of animals used.

BUDGET JUSTIFICATION

PERSONNEL: The salary levels are consistent with the prevailing job market in Chicago. Three people are necessary because of the nature of the studies plus the fact that we will be producing large batches of stroma-free hemoglobin solution for designated investigators as well as studying its properties ourselves. We are requesting an 8% salary increase.

BABOONS: Four (4) for the Brain Oxygenation Study, five (5) for the Renal Study, five (5) for the Myocardial Study and three (3) for the collaborative study. Seventeen (17) baboons at $360.00 each = $6,120.00. The average monthly charge for animal care is $140.00 for twelve (12) months, which equals $1,680.00 per year.

CLERICAL, SECRETARIAL AND EDITORIAL SERVICES:

This category will cover the costs of typing abstracts and manuscripts, mailing reprint requests and answering scientific inquiries. Further, the cost of scientific and fiscal record keeping will be covered by these expenditures.

BUDGET

JANUARY 1, 1978 -- DECEMBER 31, 1977

1.	SALARIES		
	A.	Biochemist	$ 12,981.60
		Fringe Benefits	1,947.24
		Overhead, 47.1% of Salaries and Wages	6,114.33
	B.	Biochemist	12,981.60
		Fringe Benefits	1,947.24
		Overhead, 47.1% of Salaries and Wages	6,114.33
	C.	Biochemistry Technician/Part-Time	2,700.00
		Fringe Benefits	405.00
		Overhead, 47.1% of Salaries and Wages	1,271.70
	D.	Clerical Services	4,968.00
		Fringe Benefits	745.20
		Overhead, 47.1% of Salaries and Wages	2,339.92
		Total:	$ 54,516.16
2.	SUPPLIES		
	A.	Baboons - 17 @ $360.00 each	$ 6,120.00
	B.	Animal Care, Approximately $140.00/mo.	1,680.00
	C.	Expendible Supplies	1,000.00
		Total:	$ 8,800.00
		GRAND TOTAL:	$ 63,316.16

TABLE I

DEXTRAN GROUP
% Change Due to Isoproterenal

Variable	Before Dextran Exchange	After Dextran Exchange
Peripheral Resistance	-43%	-45%
Stroke Work	+13.5%	+15%
Central Venous Pressure	+14%	+8%
Heart Rate	+36%	+28%
Cardiac Output	65%	+61%
Stroke Volume	22%	27%
Cardiac Work	56%	42%
Peak Flow	61%	56%
Mean Arterial Pressure	-6.5%	-14.5%
Systolic Arterial Pressure	4.5%	-2%
Diastolic Arterial Pressure	-16%	-19.5%

TABLE II

HEMOLYSATE GROUP
% Change Due to Isoprotesenol

Variable	Before Hemolysate Exchange	After Hemolysate Exchange
Peripheral Resistance	-43%	-30%
Stroke Work	-5%	+16%
Central Venous Pressure	+17.5%	-2%
Heart Rate	+58%	+23%
Cardiac Output	62.5%	44%
Stroke Volume	4.5%	18%
Cardiac Work	49.5%	42%
Peak Flow	46%	33%
Mean Arterial Pressure	-8%	-2%
Systolic Arterial Pressure	4%	11.5%
Diastolic Arterial Pressure	-18%	-15.5%

TABLE III

COMPARISON OF DEXTRAN VS. HEMOLYSATE GROUP

VARIABLE	BEFORE EXCHANGE TRANSFUSION		AFTER EXCHANGE TRANSFUSION	
	t	p	t	p
HR	-7.00	< .001	1.92	.073
SV	6.60	< .001	2.46	.026
CO	0.38	.710	3.10	.007
DAP	0.36	.725	- .81	.431
SAP	.03	.979	-4.58	< .001
MAP	0.26	.800	-2.94	.010
SW	2.83	.012	- .65	.528
CW	0.36	.726	.02	.984
PR	-0.05	.962	-4.93	< .001
PF	5.84	< .001	4.51	< .001
CVP	N.S.	N.S.	N.S.	N.S.

TABLE IV

COMPARISON BEFORE VS. AFTER EXCHANGE

VARIABLE	DEXTRAN GROUP		HEMALYSATE GROUP	
	t	p	t	p
HR	-2.77	.012	-4.03	.001
SV	1.59	.129	2.72	.013
CO	- .61	.550	-3.20	.005
DAP	- .82	.420	.32	.753
SAP	-2.18	.042	1.45	.164
MAP	-1.60	0.129	1.09	.289
SW	- .27	.788	3.02	.007
CW	-1.41	.176	- .62	.546
PR	- .77	.451	3.34	.003
PF	-1.37	.187	-3.16	.044
CVP	N.S.	N.S.	N.S.	N.S.